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13. ABSTRACT (Maximum 200 words)

A study of the hydrodynamics characterization of the excitation of bioluminescence was performed in collaboration with NRaD scientist Dr. James Rohr. Present research using fully developed pipe flow confirmed previous work using Couette flow that the excitation threshold for dinoflagellate bioluminescence occurred in laminar flow at a shear stress level greater than typical levels in the mixed layer. Maximum response was achieved at high laminar flow values, with no further increase for turbulent flows. A study of the spontaneous bioluminescence of the dinoflagellate Ceratocorys horrida revealed that this species exhibited circadian rhythms in both spontaneous flashing and glowing. Spontaneous light emission in dinoflagellates may be an important source of natural bioluminescence in the ocean. Several approaches tested the hypothesis that spontaneous flashing by dinoflagellates is caused by cell collisions. The results from experiments involving impaired swimming, direct observations of colliding cells, and surface to volume manipulations were not able to confirm the hypothesis. Unialgal red tide dinoflagellate diet significantly affected the total bioluminescence potential and flash intensity of two local species of heterotrophic dinoflagellates. Cannibalism was an important source of nutrition during periods of prey scarcity. Bioluminescence appears to be a sensitive indicator of energetic state in heterotrophic dinoflagellates.

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FINAL TECHNICAL REPORT

BIOLUMINESCENCE SOURCE EMISSION CHARACTERIZATION

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BIOLUMINESCENCE SOURCE EMISSION CHARACTERIZATION

LONG-TERM GOALS

Bioluminescence displays are components of fundamental organism behaviors, including predator/prey interactions and intraspecific signaling, all considered crucial in the structuring of biological communities. My long-term goals are to understand the environmental and physiological factors which regulate the emission of bioluminescence, and to determine the roles of bioluminescent displays in biological interactions.

PROJECT GOALS

The goals of this project include: (1) develop protocols for characterizing the hydrodynamic excitation of dinoflagellate bioluminescence using well-defined flow fields, (2) survey the occurrence of spontaneous bioluminescence in cultured dinoflagellates, (3) test the hypothesis that spontaneous dinoflagellate flashing is due to mechanical stimulation, and (4) investigate the use of bioluminescence as a sensitive indicator of energetic state in heterotrophic dinoflagellates.

APPROACH

Dinoflagellates, the most common sources of near-surface oceanic bioluminescence, are model organisms for examining how plankton cells respond to water motion. They are smaller in size than the Kolmogorov (eddy) scale typical of oceanic flows, can be considered passive flow tracer particles because they are weak swimmers relative to bulk flow, and the autotrophic forms are readily grown in culture.

Natural levels of *in situ* bioluminescence may originate not only from predator/prey interactions, but also from the so-called "spontaneous" bioluminescence of dinoflagellates. Although this phenomenon is known in 6 species, the mechanism responsible for spontaneous flashing is unknown. Previously unscreened species of laboratory cultured dinoflagellates were test for the occurrence of spontaneous bioluminescence. A more detailed study of the spontaneous bioluminescence in two species of dinoflagellates was performed to test the hypothesis that spontaneous flashing is caused by cell collisions.

Heterotrophic dinoflagellates are recently known to be common sources of bioluminescence in near-surface waters. Whereas the light emission of photosynthetic dinoflagellates is related to the level and amount of irradiance, the bioluminescence of heterotrophic forms should be related to their diet. However, few studies have been undertaken because of the difficulty in maintaining heterotrophic cells in culture. The effects of red tide dinoflagellate diet on the bioluminescence of two local species of *Protoperidinium* was undertaken.

TASKS ACCOMPLISHED

A study of the hydrodynamics characterization of the excitation of bioluminescence was performed in collaboration with Dr. James Rohr, NCCOSC San Diego. Initial results were presented at the 1994 Ocean Sciences meeting (Latz and Rohr, 1994) and a manuscript detailing the responses of *Gonyaulax polyedra* is being submitted for publication in *Limnology and Oceanography* (Latz et al. 1996).

A study of the spontaneous bioluminescence of the dinoflagellate *Ceratocorys horrida* was completed. This species exhibits circadian rhythms in both spontaneous flashing and glowing. The results of this study have been published in the *Journal of Phycology* (Latz and Lee, 1995).

Three approaches were used to test the hypothesis that spontaneous flashing is caused by cell collisions: (1) measure the effect of impaired swimming ability on flash rate, (2) directly observe if flash collisions stimulate flashes, (3) investigate the effect of surface to volume manipulations on spontaneous flash rate, and (4) test an encounter model predicting the rate of flashing based on cell encounters.

A study was completed on the dietary factors regulating the bioluminescence of two species of local heterotrophic dinoflagellates, *Protoperidinium divergens* and *P. crassipes*. One paper has been published on population growth and ingestion while in laboratory culture (Jeong and Latz, 1994). Our study of the effect of red tide dinoflagellate diet on bioluminescence was first presented at the ONR-sponsored 1993 Bioluminescence Symposium (Latz and Jeong, 1993); the final paper is being published in *Marine Ecology Progress Series* (Latz and Jeong, 1996).

SCIENTIFIC RESULTS

Experiments using fully developed pipe flow revealed that the excitation threshold for bioluminescence stimulation occurred in laminar flow, at a wall shear stress of approximately 2 dyn cm^{-2}. For wall shear stresses of 2-20 dyn cm^{-2} bioluminescence increased according to a power law relationship with shear stress, due primarily to an increase in the proportion of the cell population being excited, and to a lesser degree the maximum flash intensity per cell. At equivalent values of wall shear stress, there were no differences between excitation for laminar and turbulent flows . The measured bioluminescence excitation threshold is similar to previous results (Latz et al. 1994), and is several orders of magnitude greater than typical values of oceanic shear stress, although the threshold is consistent with an antipredation strategy for bioluminescence.

At least 6 species of dinoflagellates are known to produce spontaneous bioluminescence, in which light emission is unstimulated by the scientist. *Ceratocorys horrida* is only the second species in which spontaneous bioluminescence is known to exhibit diurnal rhythmicity in both flashing and glowing. *C. horrida* also exhibits a diurnal rhythm in stimulated bioluminescence. Spontaneous light emission represents only a small proportion of bioluminescence potential.

Even though dinoflagellates swim at Reynolds numbers <1 where viscous forces dominate, microscope videography reveals that cells do collide with one another. These collisions may result in the mechanical stimulation of bioluminescence. If cell swimming is a cause of "spontaneous" flashing, then inhibiting cell swimming by increased fluid viscosity should reduce the rate of spontaneous flashing. When Ficoll was used to increase viscosity, there was an expected decrease in spontaneous flash rate in *Gonyaulax polyedra* and *Ceratocorys horrida.* Polyvinylpyrrolidone (PVP) also decreased the spontaneous flash rate at 5x viscosity, but at 10x viscosity it decreased the flash rate only for *C. horrida*. Agarose increased the flash rate of *G. polyedra* at all concentrations used. Cell collisions were directly observed using a dual camera imaging system mounted on an inverted microscope, with the video of an intensified camera filming bioluminescence overlaid onto that of an infrared transmitted light view of cell swimming. Cell encounters did lead to cell flashing, although this usually occurred in only one of the cells, and not all encounters resulted in flashing. In general, experiments were not able to confirm the hypothesis that spontaneous flashes were due to cell collision.

Unialgal red tide dinoflagellate diet significantly affected total mechanically stimulable luminescence (TMSL) and flash intensity of *Protoperidinium divergens*, but not the total number of flashes produced by each cell. For all diets, TMSL was significantly correlated with maximum feeding frequency (MFF) rather than maximum population growth rate. For example, *P. divergens* displayed high levels of TMSL and MFF when fed a *Scrippsiella trochoidea* diet, even though it had a zero population growth rate with this prey. Individually isolated cells remained

viable for only a few days without food, and exhibited reduced bioluminescence, while those maintained in groups without food survived at least 16 days and maintained levels of bioluminescence similar to those under favorable prey conditions. Cannibalism observed during group maintenance without added food may have enabled large cells to feed on smaller conspecific cells and thus obtain energy for bioluminescence and survival. The results of this study suggest that energy allocation is prioritized in the following order: swimming (for grazing) > bioluminescence (for reducing predation) > growth (for increasing population).

SIGNIFICANCE

Present research confirms previous work (Latz et al. 1994) that the excitation threshold for dinoflagellate bioluminescence occurs in laminar flow at a shear stress level greater than typical levels in the mixed layer. For dinoflagellate bioluminescence, the increase in bioluminescence with increasing shear stress is due primarily to an increase in the proportion of the population that is responding. Maximum bioluminescence response is achieved at high laminar flow values, and does not further increase for turbulent flows. For an object moving through the water, turbulent flow on the body stimulates more bioluminescence than regions of laminar flow because of increased mixing. The bioluminescence signatures of specific flow fields in coastal waters is important for both surveillance and covert applications of Naval interest. Bioluminescence is a sensitive instantaneous monitor of responsiveness to fluid motion by planktonic organisms, and a powerful tool for relating organism response to small-scale fluid processes, important in marine plankton community dynamics.

PUBLICATIONS

Refereed Publications

Latz, M.I. and H.J. Jeong. 1996. Effect of red tide dinoflagellate diet on the bioluminescence of the heterotrophic dinoflagellates *Protoperidinium* spp. Marine Ecology Progress Series. In press.

Latz, M.I. and A.O. Lee. 1995. Spontaneous and stimulated bioluminescence of the dinoflagellate, *Ceratocorys horrida* (Peridiniales). Journal of Phycology 31: 120-132.

Latz, M.I. 1995. Physiological mechanisms in the control of bioluminescent countershading in a midwater shrimp. Marine and Freshwater Physiology and Behavior 26: 207-218.

Neilson, D.J., M.I. Latz, and J.F. Case. 1995. Temporal variability in the vertical structure of bioluminescence in the North Atlantic Ocean. Journal of Geophysical Research 100 (C4): 6591-6603.

Omori, M., M.I. Latz, H. Fukami, and M. Wada. 1995. New observations of bioluminescence of the pelagic shrimp *Sergia lucens* (Hansen, 1922). Marine and Freshwater Behavior and Physiology 26. In press.

Latz, M.I., J.F. Case, and R.L. Gran. 1994. Excitation of bioluminescence by laminar fluid shear associated with simple Couette flow. Limnology and Oceanography 39: 1424-1439.

Jeong, H.J. and M.I. Latz. 1994. Growth and grazing rates of the heterotrophic dinoflagellates *Protoperidinium* spp. on red tide dinoflagellates. Marine Ecology Progress Series 106: 173-185.

Case, J.F., E.A. Widder, S. Bernstein, K. Ferrer, D. Young, M.I. Latz, M. Geiger, and D. Lapota. 1993. Assessment of marine bioluminescence. Naval Research Reviews 45(2): 31-41.

Herring, P.J., M.I. Latz, N.J. Bannister, and E.A. Widder. 1993. Bioluminescence of the poecilostomatoid copepod *Oncaea conifera*. Marine Ecology Progress Series 94: 297-309.

Invited papers

Latz, M.I. 1995. Cryptic bioluminescence in the marine environment. Sensory Ecology and Physiology of Zooplankton Symposium, Honolulu, Hawaii.

Omori, M., H. Fukami, and M.I. Latz. 1995. Confirmation and measurement of bioluminescence of the pelagic shrimp *Sergia lucens*. Sensory Ecology and Physiology of Zooplankton Symposium, Honolulu, Hawaii.

Latz, M.I. 1994. Cryptic bioluminescence in the midwater environment. Seminar to Tokyo University of Fisheries, Tokyo, Japan.

Latz, M.I. 1994. Cryptic bioluminescence in the midwater environment. Seminar to Tokai University, Shizuoka, Japan.

Latz, M.I. 1993. Excitation mechanisms of plankton bioluminescence. ONR Bioluminescence Symposium. Maui, Hawaii.

Contributed papers

Latz, M.I. and J. Rohr. 1994. Are plankton indifferent to the nature of shear stress? Evidence from bioluminescence excitation studies. Eos 75: 184.

Latz, M.I. and H.J. Jeong. 1993. Effect of dinoflagellate diet and starvation on the bioluminescence of the heterotrophic dinoflagellate, *Protoperidinium divergens*. ONR Bioluminescence Symposium, Maui.

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